FABAD J. Pharm. Sci., 22, 1-7, 1997

RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

Synthesis, Structural and Conformational Analysis of New Thiazolo [3,2-a] pyrimidine Compounds by Nuclear Magnetic Resonance Techniques and High Pressure Liquid Chromatography

Hülya AKGÜN*, Birsen TOZKOPARAN*, Mevlüt ERTAN*°

Synthesis, Structural and Conformational Analysis of New Thiazolo [3,2-a] pyrimidine Compounds by Nuclear Magnetic Resonance Techniques and High Pressure Liquid Chromatography

Summary : *New 1,4-dihydropyridine analog compounds having some 2,3-dihydro-thiazolo[3,2-a]pyrimidine ring were synthesized. Conformational analysis of the compounds were verified with ^{1}H, ^{13}C, H,H-COSY, H,C-COSY, DEPT 90° and 135° NMR spectroscopic methods and HPLC techniques using chiral and achiral columns.*

Key words: *Thiazolo [3,2-a] pyrimidine, conformational analysis, H,H-COSY, H,C-COSY, DEPT 90° and 135°, HPLC*

Received : 5.7.1996
Revised : 9.10.1996
Accepted : 9.10.1996

Yeni Tiyazolo[3,2-a]pirimidin Türevlerinin Sentezleri, Nükleer Manyetik Rezonans Teknikleri ve Yüksek Basınçlı Sıvı Kromatografisi ile Yapısal ve Konformasyonel Analizleri

Özet : *Bu çalışmada 2,3-dihidro-tiyazolo[3,2-a]pirimidin yapısında yeni 1,4-dihidropiridin benzeri bileşikler sentezlenmiştir. Bileşiklerin konformasyonel analizleri ^{1}H, ^{13}C, H,H-COSY, H,C-COSY, DEPT 90° ve 135° NMR spektroskopik metodları ve kiral-akiral kolonların kullanıldığı HPLC teknikleri kullanılarak yapılmıştır.*

Anahtar kelimeler : *Tiyazolo[3,2-a]pirimidin, konformasyonel analiz, H,H-COSY, H,C-COSY, DEPT 90° ve 135°, HPLC*

1. Introduction

1,4-Dihydropyridine derivatives possessing calcium antagonistic action in the cardiovascular system have attracted much synthetic attention over the past 20 years[1-3]. Calcium antagonists decrease influx of calcium ions through plasma membrane channels and thus dilate vascular smooth muscle and alleviate the force of cardiac muscle contraction[4].

In order to produce more potent vasodilating compounds several modifications have been made on 1,4-dihydropyridine derivatives especially on Nifedipine I by changing the ester groups and/or the substituents on phenyl nucleus[5-6]. Our research program on calcium antagonist have focused on the 2-oxo(or thioxo)1,2,3,4-tetrahydropyrimidines and their derivatives which can be regarded as aza-analogs of nifedipine related dihydropyridines. In our previous studies, we synthesized some 2-thioxo-1,2,3,4-tetrahydropyrimidines II and tested their Ca-antagonistic[7,8] and antiaggregating effects in vitro[8].

It was also reported that conformational differences of 1,4-dihydropyridine ring are important for agonist-antagonist response[9].To supply the conformational rigidity, some thiazolo[3,2-a]pyrimidines

* Hacettepe University, Faculty of Pharmacy, Dept. of Pharmaceutical Chemistry, 06100, Ankara-TURKEY.
° Correspondence : Hacettepe University, Faculty of Pharmacy, Dept. of Pharmaceutical Chemistry, 06100, Ankara-TURKEY.

III were prepared starting from 2-thioxo-1,2,3,4-tetrahydropyrimidines[10,11]. These compounds III showed greater biological activity in comparison with the starting compounds II[11]. The interesting pharmacological results obtained in the study of fused heterocyclic structures prompted us to prepare new thiazolo[3,2-a]pyrimidine derivatives which are expected to have calcium antagonist activity. Here we described the synthesis and absolute configuration of new thiazolo[3,2-a]pyrimidine derivatives using NMR experiments and HPLC .

2. Experimental

2.1. Chemistry

2.1.1. Devices and Chemicals

Melting points were determined with Thomas-Hoover Capillary melting point apparatus and uncorrected.

The IR-spectra were recorded in KBr pellets on a Perkin Elmer FT IR 1720X spectrophotometer.

1D Experiment : ^{1}H, ^{13}C, off-resonance spectra of the compounds, were obtained at 300 MHz for ^{1}H and 75.5 mHz for ^{13}C on Bruker AM-300 ($CDCl_3$) TMS as an internal reference. The proton $\pi/2$ pulse was 4.7 µs. Typical conditions for proton spectra were spectral width of 3597 Hz with block size of 32 K 64 scans of acquisiton. Standard Bruker microprograms were used for the DEPT 90° and 135° experiments.

2D experiment : For the H,H-COSY experiment a 512 x 2K matrix was collected over a 1799 Hz using standart COSY AU. microprogram. For H,C-COSY experiment a 256 x 2K matrix was collected over 1799 Hz using standard XHCORR.AU Bruker microprogram. All chemical shifts are expressed in ppm downfield from TMS.

High Performance Liquid Chromatography Apparatus: A Knauer liquid chromatography was equipped with a UV absorbance detector operated at 254 nm. Separation were performed using on line coupling of an achiral analytical column, Merck LiChrospher 100 Rp-18 (particle size 5 µm) and a chiral analytical column chiracel-OD 250x4. The detector signal was recorded and reported as peak areas on a plotter recorder. The solvent used were HPLC grade.

Elementary analysis were performed by the Scientific and Technical Research Council of Turkey.

Ethyl 2,3-dibromopropionate, triethylamine (TEA), dimethylformamide (DMF) were from Aldrich.

(±) 1,2,3,4-Tetrahydro-6-methyl-4-(3-nitrophenyl)-2-thioxo-5-pyrimidinecarboxylic acid esters II were prepared by the method of [7,8].

2.1.2. (±) 2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylates (IVa-b, Va-b).

0.002 Mol (±) 1,2,3,4-tetrahydro-6-methyl-4-(3-nitrophenyl)-2-thioxo-5-pyrimidine-carboxylic acid esters II was dissolved in 0.006 mol triethylamine and 4 ml N,N-dimethylformamide. 0.002 Mol ethyl 2,3-dibromopropionate was added to the reaction mixture by cooling under N_2 atmosphere in 1 h. Then, the mixture was kept for 24 h. at room temperature by stirring and poured into the crushed ice.The resulting aqueous solution was extracted with dichloromethane. Organic layer was washed with saturated NaCl solution and water, then dried over Na_2SO_4. After removal of the solvent in vacuo, oily residue was obtained. Diastereomers are separated by column chromatography (silica gel 60, particle size 0.063-0.200 mm ; n-hexane : ethyl acetate (1:1)).

2.1.2.1. Methyl 2-carbethoxy-5-(3-nitrophenyl)-7-methyl-2,3-dihydro-5H-thiazolo-[3,2-a]pyrimidine-6-carboxylate (IVa, IVb)

Compounds IVa and IVb were prepared according to general procedure. Yield: 0.4 g (51 %). The mixt. of IVa and IVb $C_{18}H_{19}N_3O_6S$ (405.43)

Calcd. : C 53.33 H 4.72 N 10.36.

Found : C 53.91 H4.93 N 10.10.

Isomers were separated by column chromatography and oily residue solidified with diethyl ether.

For IVa, m.p. 119-121°C. IR (cm^{-1}) : 2929 (C-H), 1737,1697 (C=O), 1605,1528 (C=C, C=N) .^{1}H and ^{13}C NMR results are shown in table 1.

For IVb, m.p. 161-163 oC. IR (cm-1) : 2929 (C-H), 1745,1672 (C=O), 1598,1534 (C=C, C=N).^{1}H and ^{13}C NMR results are shown in table 1.

2.1.2.2. Ethyl 2-carbethoxy-5-(3-nitrophenyl)-7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate (Va, Vb)

Compounds Va and Vb were also prepared according to general procedure. Yield 0.7 g (84 %). The mixt. of Va and Vb $C_{19}H_{21}N_3O_6S$ (419.45)

Table 1 : Chemical shift assignments of the compounds IVa and IVb

	IVa		IVb	
Carbon	^{13}C	^{1}H	^{13}C	^{1}H
2	42.65	4.25(dd;1H,J_{2a-3a}:8.6 Hz; J_{2a-3e} : 4.5 Hz)	41.73	4.05-4.10(m;1H)
3	53.14	3.70-3.85 (m;2H)	53.36	3.45(dd;1H,J_{3a-3e} : 11.1 Hz; J_{3a-2a} : 8.5 Hz) 4.05-4.1 (m;1H)
5	60.15	5.50 (s;1H)	60.04	—
6	103.51	—	103.45	—
7	148.95*	—	149.15*	5.55 (s;1H)
8a	165.39*	—	165.27*	—
9	143.87*	—	143.76*	—
10	123.29*	8.10-8.20 (m;1H)	123.21*	8.15-8.20 (m;1H)
11	156.89*	—	157.02*	—
12	124.25*	8.10-8.20 (m;1H)	124.26*	8.15-8.20 (m;1H)
13	130.62*	7.50-7.60 (m;1H)	130.85*	7.50-7.60 (m;1H)
14	134.57*	6.65-6.75 (m;1H)	134.34*	7.70-7.75 (m;1H)
1'	167.25*	—	167.17*	—
2'	63.35	4.08 (q;2H)	63.49	4.25 (q;2H)
3'	14.57	1.15 (t;3H, J: 7 Hz)	14.67	1.25 (t;3H)
4'	23.91	2.30 (s;3H)	23.96	2.35 (s;3H)
5'	51.87	3.55 (s;3H)	51.85	3.60 (s;3H)
6'	169.28*	—	169.98*	—

* These values may be interchanged

Table 2 : Chemical shift assignments of the compounds Va and Vb

	Va		Vb	
Carbon	^{13}C	^{1}H	^{13}C	^{1}H
2	42.61	4.05(dd;1H,J_{2a-3a}:8.6 Hz; J_{2a-3e} : 4.3 Hz)	41.74	4.00-4.10 (m;1H)
3	53.14	3.75-3.98 (m;2H)	53.38	3.5(dd;1H,J_{3a-3e} : 11.1 Hz; J_{3a-2a} : 7.5 Hz) 4.05 (m;1H)
5	60.21	5.55 (s;1H)	60.15	—
6	103.64	—	103.64	—
7	149.15*	—	149.06*	5.55 (s;1H)
8a	165.20*	—	165.13*	—
9	144.01*	—	143.94*	—
10	123.40*	8.15-8.17 (m;1H)	123.20*	8.15-8.16 (m;1H)
11	156.95*	—	156.80*	—
12	124.21*	8.15-8.17 (m;1H)	124.25*	8.15-8.16 (m;1H)
13	130.61*	7.53-7.56 (m;1H)	130.85*	7.53-7.56 (m;1H)
14	134.65*	7.71-7.74 (m;1H)	134.34*	1.69-7.72 (m;1H)
1'	166.73*	—	166.64*	—
2'	60.83	3.98-4.01 (m;2H)	60.82	4.09-4.19 (q;2H)
3'	14.57	1.15 (t;3H)	14.70	1.15 (t;3H)
4'	23.89	2.36 (s;3H)	23.88	2.37 (s;3H)
5'	14.85	1.15 (t;3H)	14.85	1.23 (t;3H)
6'	63.34*	3.98-4.02 (m;2H)	63.52	4.15-4.30 (m;2H)
7'	169.31*	—	170.01*	—

* These values may be interchanged

Table 3 : NOE experimental results of IVa and IVb

NOE of IVa

5H	to	3H (s), o, o-phenyl (s)
2H	to	3H(w), OCH_2 (s)
7-CH_3	to	OCH_3 (s)

NOE of IVb

5H	to	$3H_a$ (s), $3H_e$ (w), OCH_2 (w), o, o-phenyl (s)
2H	to	$3H_a$ (w), $3H_e$ (s), OCH_2 (s), 5H (w)
7-CH_3	to	OCH_3 (s)

w : week, s : strong

Table 4 : HPLC results of the compounds IV and IVb

Compound	Column	Mobile Phase	Rt
The mixture of IVa and IVb	Licrospher-100 Rp-18 (Merck)	60 / 40 MeOH / H_2O	11.0, 13.4
The mixture of IVa and IVb	Licrospher-100 Rp-18 (Merck)	50 / 50 MeOH / H_2O	30.4, 35.5
IVa	Licrospher-100 Rp-18 (Merck)	60 / 40 MeOH / H_2O	11.0
IVb	Licrospher-100 Rp-18 (Merck)	60 / 40 MeOH / H_2O	13.4
The mixture of IVa and IVb	Chiracel OD	95 / 5 n-Hexane / EtOH	21.6, 23.0, 26.9, 29.4
The mixture of IVa and IVb	Chiracel OD	90 / 10 n-Hexane / EtOH	34.6, 36.5, 44.7, 50.5
IVa	Chiracel OD	95 / 5 n-Hexane / EtOH	26.9, 29.5
IVb	Chiracel OD	95 / 5 n-Hexane / EtOH	21.9, 23.2

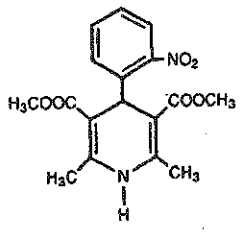
Table 5 : HPLC results of the compounds Va and Vb

Compound	Column	Mobile Phase	Rt
The mixture of Va and Vb	Licrospher-100 Rp-18 (Merck)	60 / 40 MeOH / H_2O	17.4, 20.8
The mixture of Va and Vb	Licrospher-100 Rp-18 (Merck)	50 / 50 MeOH / H_2O	34.6, 40.5
Va	Licrospher-100 Rp-18 (Merck)	60 / 40 MeOH / H_2O	17.5
Vb	Licrospher-100 Rp-18 (Merck)	60 / 40 MeOH / H_2O	19.8
The mixture of Va and Vb	Chiracel OD	92 / 8 n-Hexane / EtOH	18.1, 19.1, 21.8, 25.9
The mixture of Va and Vb	Chiracel OD	94 / 6 n-Hexane / EtOH	28.9, 30.2, 36.0, 44.4
Va	Chiracel OD	92 / 8 n-Hexane / EtOH	21.4, 25.4
Vb	Chiracel OD	92 / 8 n-Hexane / EtOH	17.8, 19.0

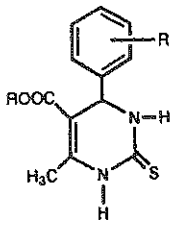
Calcd. : C 54.41 H 5.05 N 10.02.
Found : C 54.74 H 5.11 N 9.98.

Isomers were separated by column chromatography and oily residue solidified with diethyl ether.

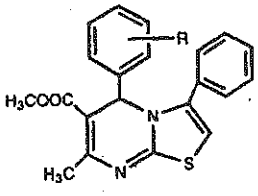
For Va, m.p. 124-125 °C. IR (cm^{-1}) : 2983 (C-H), 1744,1694 (C=O), 1601,1537 (C=C, C=N). ^1H and ^{13}C NMR results are shown in table 2.



I



II



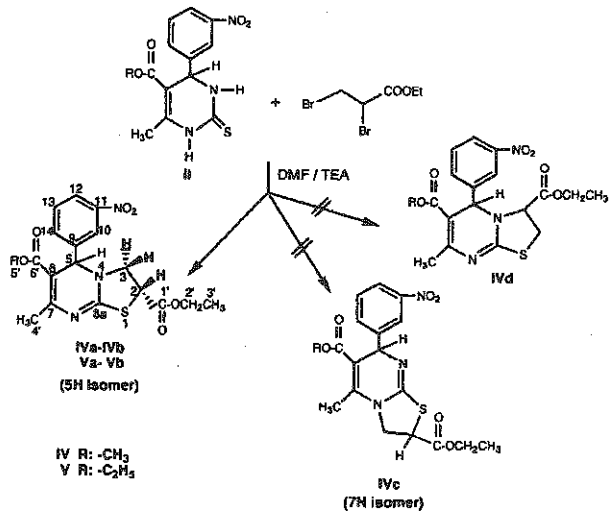
III

For Vb, m.p. 138-140 °C. IR (cm^{-1}): 2979 (C-H), 1742,1693 (C=O), 1602,1527 (C=C, C=N). ^1H and ^{13}C NMR results are shown in table 2.

3. Results and Discussion

Thiazolo[3,2-a]pyrimidine derivatives having two optically active center at C2 and C5 (IVa-b, Va-b), were synthesized by the reaction of II with ethyl 2,3-dibromopropioanate (Scheme1).

Compound II can be considered as a cyclic thiourea derivative, and therefore can react with various dielectrophiles to yield fused pyrimidines. Several cyclization products may be expected (IVa-IVd, Va-Vd). For both pathways examples are known, deriving from II (IVa, IVc) [12,13]. It was reported that the N(3) nitrogen is more reactive towards electrophiles than the N(1) nitrogen due to the ester group in the 5 position of the pyrimidine ring[14]. In the light of previous related work, it is likely that ethyl 2,3-dibromopropionate attacked II regiospecifically at the N(3) nitrogen atom to yield 5H isomers where the carboxylic acid ester group is in either position 2 or 3 (IVa, IVd)[10,14,15]. NOE experimental results, are given in Table 3; and NOESY data supported these assignments.



Scheme 1.

Off resonance ^{13}C, DEPT 90° and DEPT 135° spectra of the target compounds (IVa-IVb) were quite similar. DEPT 90° and DEPT 135° spectrum of IVa established two CH peaks at 42.65 and 60.15 ppm and two CH_2 peaks at 53.14 and 63.35 ppm. The peak at 42.65 ppm was assigned to C2 and the one at 53.14 ppm to C3. These results indicate that C2 and C3 were next to S and N atoms, respectively. It is interesting to note that compound IVd was not obtained under these reaction conditions. H,H-COSY and H,C-COSY spectra of IVa and IVb support these results.

On the other hand the ^1H NMR spectrum of the IVa and IVb differed in the interval 3.50 to 4.50 ppm (Table 1). In the ^1H NMR spectrum of IVa, the dd at 4.25 ppm arises from C2-H coupled with C3-H protons (J_{2a-3a} = 8.6 Hz; J_{2a-3e} = 4.5 Hz). The multiplet at 3.70-3.85 ppm results from C3-H protons coupled with C2-H protons. These results indicated that 2-H proton on C2 is axial whereas 2-$COOC_2H_5$ is oriented equatorially. In the ^1H NMR spectrum of

IVb, one of 3-H protons appeared at 3.45 ppm as a dd (J_{3a-3e} = 11.1 Hz; J_{3a-2a} = 8.5 Hz) whereas the other 3-H resonance was observed at 4.05-4.10 ppm as a multiplet. C2-H proton coupled with C3-H protons also arises at 4.05-4.10 ppm as multiplet. Therefore, 2-H proton on C2 is axial and 2-$COOC_2H_5$ is again oriented equatorially. C2 center of the these compounds (IVa and IVb) may have the S and R absolute stereochemistry, respectively (Fig. 1). The conformation of the five membered thiazolo ring has two alternative enantiomeric forms in the same envelope. Since the molecule contains two optically active centers (C2 and C5) we separated diastereomer pairs as IVa and IVb. However Antolini et al[16] reported the X-ray molecular structure of 7-amino-2,3-dihydro-2-phenylthiazolo[3,2-a]pyrimidin-5-one.

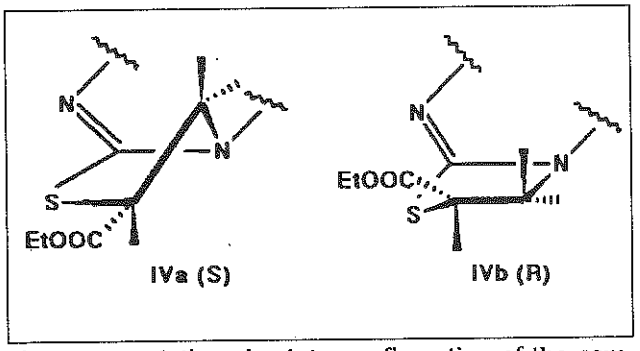


Fig. 1. Speculative absolute configuration of the compounds IVa and IVb.

They claimed that the structure consisted of two enantiomeric forms in the same envelope (E) conformation of the five-membered thiazole ring. The out of plane C2 atom and its equatorially bonded substituent (phenyl) are distributed over two alternative positions. These conclusions support our results.

Compounds Va and Vb were assigned in a similar manner and the assignments are also presented in Table 2.

In an attempt to develop an enantioselective separation of compounds IVa, IVb and Va, Vb we used HPLC under the chromatographic condition described below. In the study the enantiomers of IVa , IVb and Va , Vb were resolved. During the development of the separation, two peaks were observed when an achiral column was used . The ratio of diastereomer pairs of IV and V (IVa(Va) / IVb(Vb)) were determined as 1/3 and 1/2, respectively (Fig. 2). Four peaks, however, resulted when a chiral column was used. Experimental conditions and Rt values of the compounds were shown in Table 4,5 . It is obvious that diastereomeric pairs of the molecules can be identified by NMR, the enantiomeric pairs of the compounds can only be resolved by HPLC analysis .

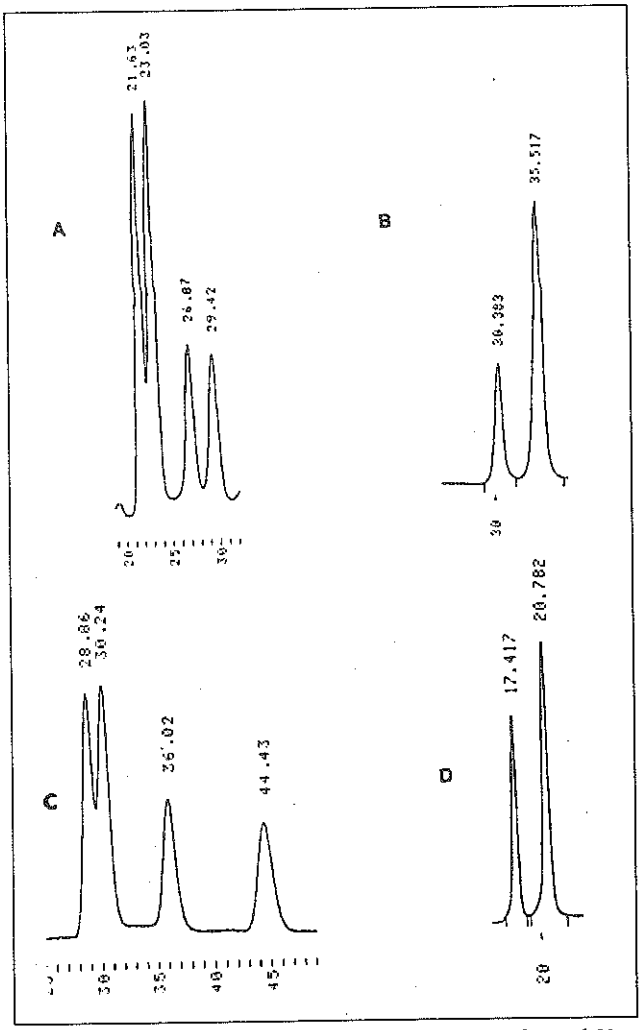


Fig. 2. Separation of the compounds IVa, IVb and Va, Vb by HPLC : **A** Separation of the mixture of IVa and IVb on Chiracel OD using n-hexane/EtOH (95/5). **B** Separation of the mixture of IVa and IVb on Licrospher 100 Rp-18 using MeOH/H_2O (50/50). **C** Separation of the mixture of Va and Vb on Chiracel OD using n-hexane/EtOH (94/6). **D** Separation of the mixture of Va and Vb on Licrospher 100 Rp-18 using MeOH/H_2O (60/40).

References

1. Bossert, F. , Vater, W. , Dihydropyridine, eine neue Gruppe stark wirksamer Coronartherapeutika, *Naturwissenschaften* , 58, 578 1971.
2. Grün, G., Fleckenstein, A., Die elektromechanische Entkoppelung der glatten Gefaβmuskulatur als Grundprinzip der Coronardilatation durch 4-(2'-Nitrophenyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarbonsaure-dimethylester (BAY a 1040, Nifedipine), *Arzneim.-Forsch.,* 22, 334-344 1972.
3. Godfraind, T., Miller, R., Wibo, M., Calcium Antagonism and Calcium Entry Blockade, *Pharmacol. Rev.*, 38, 321-416 1986.
4. Vater,W., Kroneberg, G., Hoffmeister, F., Kaller, H., Meng, K., Oberdorf, A., Puls, W., Schlobmann, K., Stoepel, K., Zur Pharmacologie von 4-(2'-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarbonsaure-dimethylester (Nifedipine, Bay a 1040) *Arzneim.-Forsch.*, 22, 1-14 1972.
5. Iwanami, M., Shibanuma, T., Fujimoto, M., Kawai, R., Tanazawa, K., Takenaka, T., Takahashi, T., Murakami, M., Synthesis of New water-soluble Dihydropyridine Vasodilators, *Chem. Pharm. Bull.*, 27, 1426-1440 1979.
6. Travis, J., Salvesen, G., Human Plasma Proteinase Inhibitors, *Ann. Rev. Biochem.*, 52, 655-709 1983.
7. Ertan, M., Balkan, A., Saraç, S.,,Uma, S., Renaud, J.F., Rolland, Y., Synthesis and Calcium Antagonistic Activity of Some New 2-Thioxo-1,2,3,4 - tetra-hydropyrimidine Derivatives, *Arch. Pharm.*, 324, 135-139 (1991).
8. Ertan, M., Balkan, A.,Saraç, S. , Uma, S., Rubseman, K., Renaud, J.F., Synthesis and Biological Evaluations of Some 2 - Thioxo - 1,2,3,4 - tetrahydropyrimidine Derivatives, *Arzneim.-Forsch.*, 41(II), 725-727 1991.
9. Fossheim, R., Crystal Structure of Dihydropyridine Ca^{2+} Antagonist Felodipine. Dihydropyridine Binding Prerequisites Assessed from Crystallographic Data, *J. Med. Chem.*, 29(2), 305-307 1986.
10. Balkan, A.; Ertan, M.; Burgemeister,Th., Synthesis and Structural Evaluations of Thiazolo [3,2-a]pyrimidine Derivatives, *Arch. Pharm.*, 325, 499-501. 1992.
11. Balkan, A., Uma,S., Ertan, M., Wiegrebe,W., Thiazolo [3,2-a]pyrimidine Derivatives as Calcium Antagonists, *Pharmazie*, 47, 687-688 1992.
12. Liu, K. C., Chern, J.-W., Yen, M.-H., Lin, Y.-O., Synthese und antihypertensive Aktivitat einiger 2,3-Dihydro-5H-thiazolo[2,3-b]chinazolin Derivate, *Arch. Pharm.*, 316, 569-571 1983.
13. Gill, N., Ralhan, N.K., Sachdev, H.S.,Narang, K.S., Studies in Thiazolopyrimidines.I. A case of Michael retrogression, *J. Org. Chem.*, 26, 968-970 1961.
14. Kappe, C. O., Roschger, P., Synthesis and Reactions of "Biginelli-Compounds". Part I, *J. Heterocyclic Chem.*, 26, 55-63 1989.
15. Takamizawa, A., Hirai, K., Ishiba, T., Matsumoto, Y., Studies on Pyrimidine Derivatives and Related Compounds. XLIV. Synthesis of Thiazolo[3,2-a]_ pyrimidine Derivatives, *Chem. Pharm. Bull.*, 15 (6),731-739 1967.
16. Antolini, L.,Schenetti, L., Rinaldi, M., Pecorari, P., Structural Studies of Pyrimido[2,1-b][1,3]thiazine and Thiazolo[3,2-a]pyrimidine rings: an X-Ray and Multinuclear (^{1}H, ^{13}C, ^{15}N) *Approach J. Chem. Research*(S), 164-165 1994.